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THE LIBERATION OF HISTAMINE BY CERTAIN ORGANIC BASES

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Histamine is liberated from mammalian tissues by a variety of agents, most of which are of a complex nature chemically. They include antigens (in sensitized animals), peptone, trypsin, lysolecithin, animal venoms and certain bacterial toxins (for references see Feldberg, 1941). In addition, several basic drugs of relatively simple structure have been reported to liberate histamine. The release of histamine from skeletal muscle by curare alkaloids (Alam, Anrep, Barsoum, Talaat & Wieninger, 1939; Schild & Gregory, 1947) and by strychnine (Schild & Gregory, 1947) is well established; and that adrenaline has a similar action has been reported by Eichler & Barfuss (1940) and by Staub (1946).

In the course of some pharmacological tests on licheniformin, an antibiotic base extracted from *Bacillus licheniformis* by Callow, Glover, Hart & Hills (1947), we found that the characteristic lowering of the cat's blood pressure produced by the substance was due to the liberation of histamine. Further investigation showed that many other organic bases produced a depressor effect of the same kind, and for the same reason.

A preliminary report of the results has already been made (MacIntosh & Paton, 1947).

METHODS

Most of our experiments were carried out on cats or dogs. Cats were usually anaesthetized with chloralose; a few were anaesthetized with ether, or were decerebrated under ether. Dogs were anaesthetized with sodium barbitone, sodium phenobarbitone or a chloralose-urethane (1:10) mixture. Blood pressure was recorded in the usual way from a carotid artery, the anticoagulant fluid being either saturated Na_2SO_4 solution, or more usually 0·9 % NaCl containing heparin. Blood samples for pharmacological tests were taken only in experiments in which no blood-pressure record was made; they were withdrawn from a carotid artery through a steel cannula filled with 1 % sodium heparin solution, into a syringe containing 0·05 c.c. of the same solution for each c.c. of blood. Plasma for pharmacological tests was obtained by centrifuging such blood samples without delay. We found that normal plasma so collected had no depressor activity when reinjected. Plasma samples and extracts were tested on the isolated ileum of the guinea-pig, suspended in Locke's solution containing 0·004 % $MgCl_2$; the volume of the bath was 20 c.c. For some tests, atropine sulphate (4×10^{-7}) or Neoantergan maleate ('Anthisan', May & Baker: 10^{-9}) was added to the fluid in the bath.

Other procedures are described in the text. Doses and concentrations of histamine are stated in terms of the base.

RESULTS

Experiments with licheniformin

Our first experiments were made with preparations of 'licheniformin hydrochloride' supplied by Dr R. K. Callow. The material so designated appears to be a mixture of basic compounds of predominantly polypeptide nature (Callow & Work, 1948). The preparations used by us were probably not of identical composition, but they were qualitatively similar in action.

The delayed depressor effect. In a cat anaesthetized with chloralose, the first effect on the arterial pressure of a small intravenous dose (e.g. 1–2 mg./kg.) of licheniformin hydrochloride is a rapid fall which closely resembles that produced, for instance, by a small dose of histamine (Fig. 1). But whereas the depressor effect of substances such as histamine or acetylcholine becomes evident a few seconds after the injection, that of licheniformin always has a longer latency, usually about 20–25 sec. Once it has begun, however, the fall in blood pressure with licheniformin may be just as rapid as with histamine. The depressor effect of such a dose of licheniformin is sometimes transient, but more often the initial level of blood pressure is not regained for several minutes. With a somewhat larger dose (e.g. 4 mg./kg.) the fall of arterial pressure is more severe and prolonged, and a yet higher dose (e.g. 10 mg./kg.) produces lasting circulatory shock; but the characteristic delay in the appearance of the depressor effect is still observed whatever the amount injected.

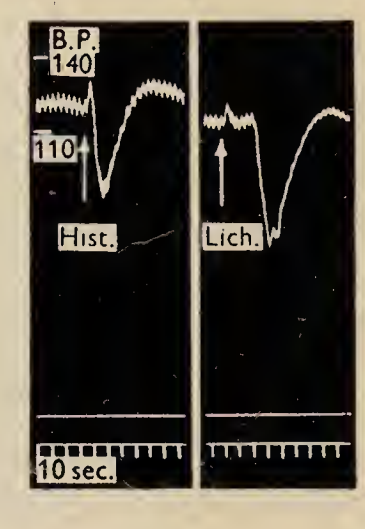


Fig. 1. Cat, chloralose: blood pressure. Hist., injection of 0·2 μg./kg. of histamine. Lich., injection of 0·95 mg./kg. of licheniformin hydrochloride.

In the cat anaesthetized with ether, or decerebrated, licheniformin sometimes causes a small immediate fall of blood pressure, which is followed by, or merges into, a steeper fall occurring after the usual latency.

The depressor action of licheniformin is unaffected by atropinization or by section of the vagi. The heart rate is initially unaffected, though cardio-acceleration, presumably reflex or due to circulating adrenaline, may be seen during the recovery phase; and the heart continues to beat strongly even when the blood pressure has been reduced to a low level by a large dose of licheniformin. That the site of the depressor action is predominantly the peripheral vessels is readily shown. The fall of arterial pressure is accompanied by an increase in limb volume (Fig. 2); and when the drug is injected alternately into the right and left auricle, the fall in blood pressure is somewhat greater in the latter case, and begins 2–3 sec. earlier (Fig. 3). In the cat the depressor effect of a small dose of licheniformin is reproducible only if the injections are spaced 10 min. or more apart. With more frequent injections, the second usually evokes

a bigger response than the first, and a dose which was initially below threshold may produce a large effect when repeated (Fig. 4). Even when the injections are timed so as to avoid this phenomenon the slope of the dose-response curve

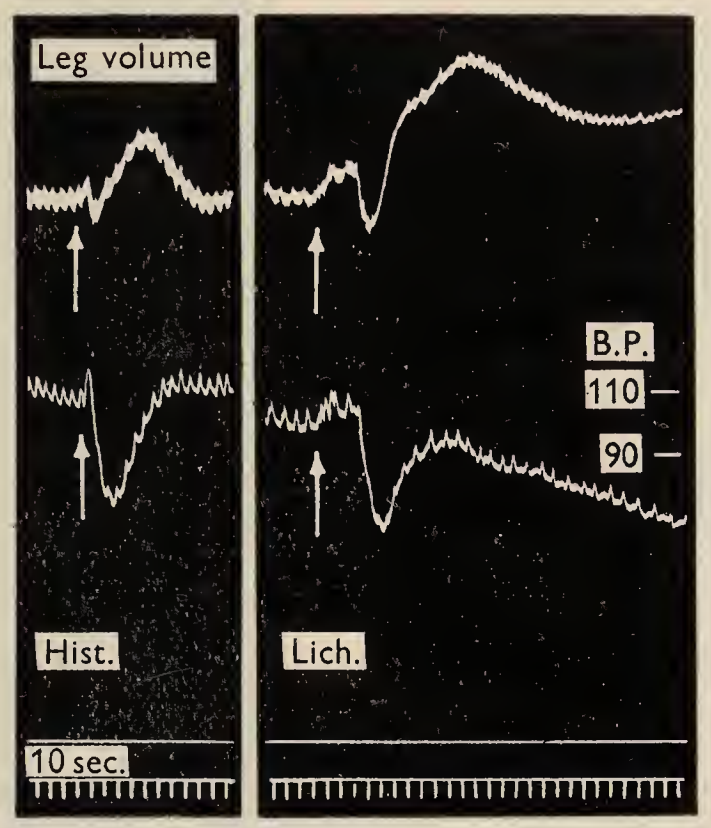


Fig. 2. Cat, chloralose: hindlimb volume and blood pressure. Hist., injection of 0·8 μg./kg. histamine. Lich., injection of 4 mg./kg. licheniformin hydrochloride.

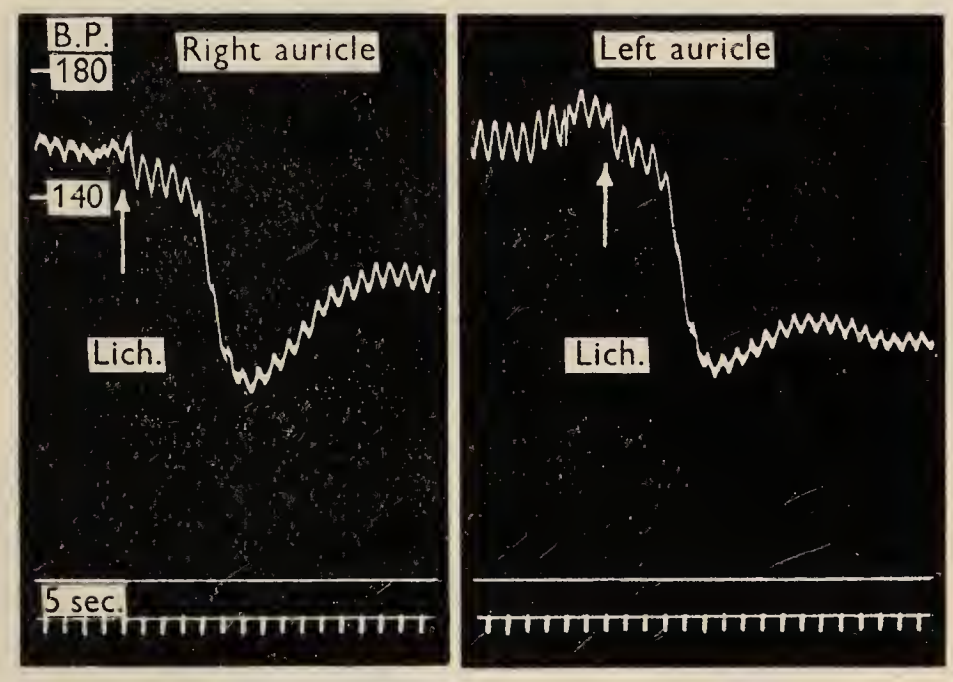


Fig. 3. Cat, chloralose, chest opened, artificial respiration. Blood pressure. Injection of 1·1 mg./kg. of licheniformin hydrochloride into right and left auricle respectively.

is found to be peculiarly steep. Thus, in one experiment, 1·0 mg. of licheniformin had no perceptible effect on the arterial pressure, but 1·5 mg. produced a fall of 45 mm. Hg. In animals whose blood pressure has been much reduced as the result of previous treatment with licheniformin, the form of the depressor effect is somewhat modified, the fall being smaller and less abrupt, and the latency longer.

The mechanism of the depressor effect. Its long latency and other peculiarities suggested that the depressor effect might be due, not to a direct action of licheniformin itself on the vessels, but to the formation or liberation, as a result of its presence in the blood, of some vasodilator substance. We therefore tested the effect of incubating licheniformin with heparinized cat's blood. The reinjection of such blood, however, produced only a delayed depressor effect, such as is produced by licheniformin itself. Contact between the drug and blood thus appeared to give rise to no new depressor substance, but it was possible that contact between the drug and the tissues might do so; the next step, therefore, was to test for depressor activity the blood of a cat which had received licheniformin. For this purpose arterial blood was withdrawn and heparinized (the heparin was free from depressor substances), and immediately injected into the vein of a second cat whose blood pressure was being recorded. The blood pressure of the donor cat was not recorded, lest the results should be complicated by the entry of anticoagulant fluid into the circulation. This experiment, in which the dose was 10 mg./kg. of licheniformin hydrochloride, gave a clear result (Fig. 5). Two c.c. of blood, withdrawn from the donor before the injection, had no effect on the blood pressure of the recipient, but the same volume of blood withdrawn 1 min. after the injection produced a sharp transient lowering of the recipient's blood pressure. This depressor effect had only a brief latency (6–8 sec.) and was, therefore, not due to licheniformin itself, which in any case could hardly have been present in significant quantity. Later blood samples from the donor cat showed diminishing depressor activity.

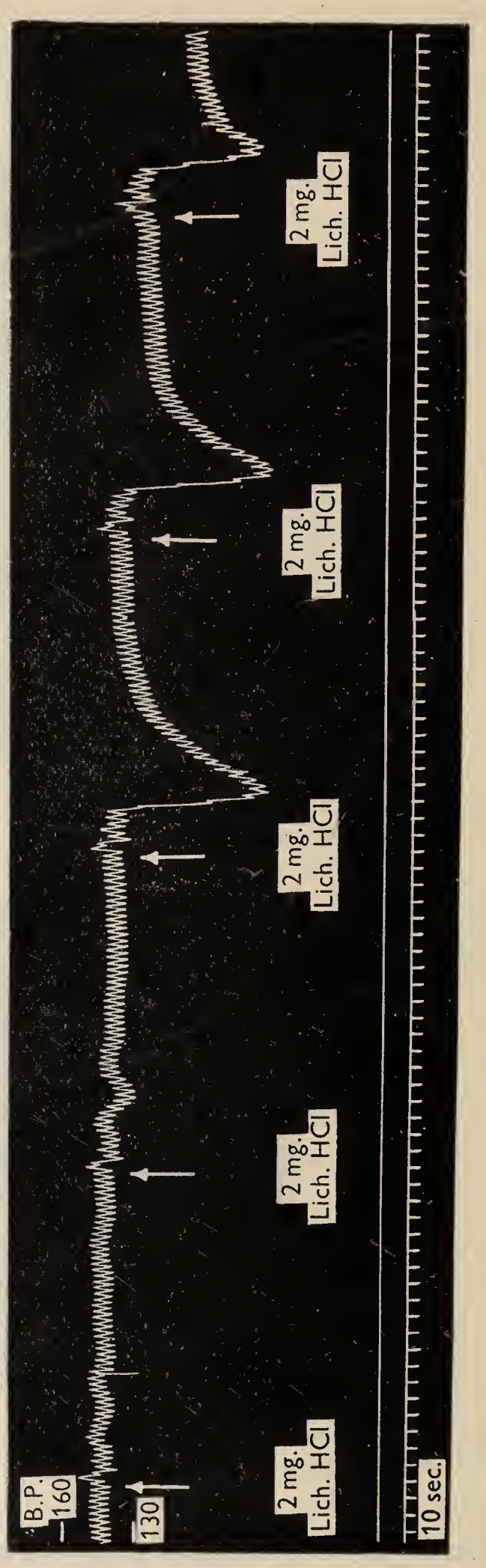


Fig. 4. Cat, chloralose: blood pressure. At 3 min. intervals, injection of 0·65 mg./kg. of licheniformin hydrochloride.

Identification of the depressor substance. Further tests were carried out with heparinized plasma instead of whole blood, which produces non-specific pressor

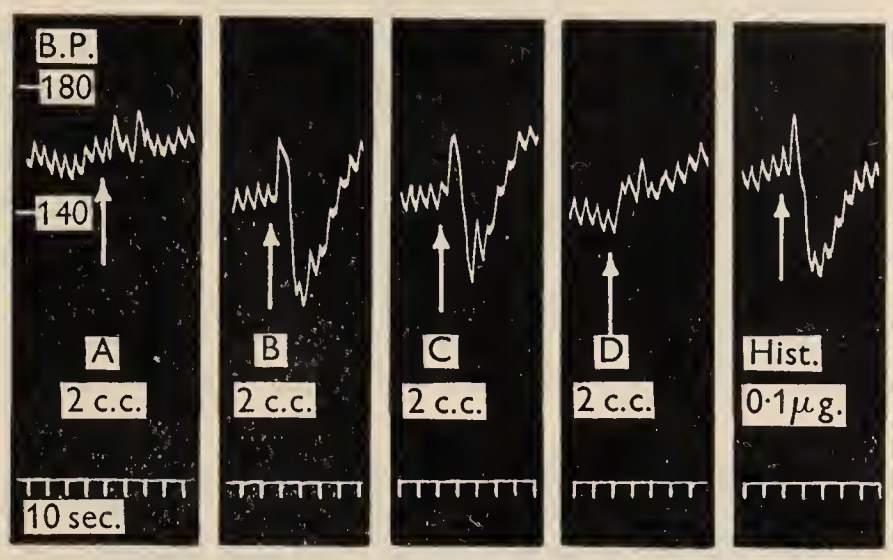


Fig. 5. Cat, chloralose: blood pressure. Injection of heparinized arterial blood samples from a second cat: A, sample obtained before injection of licheniformin hydrochloride (10 mg./kg.); B, C and D, samples obtained 1, 13 and 35 min. after the injection. Hist., histamine.

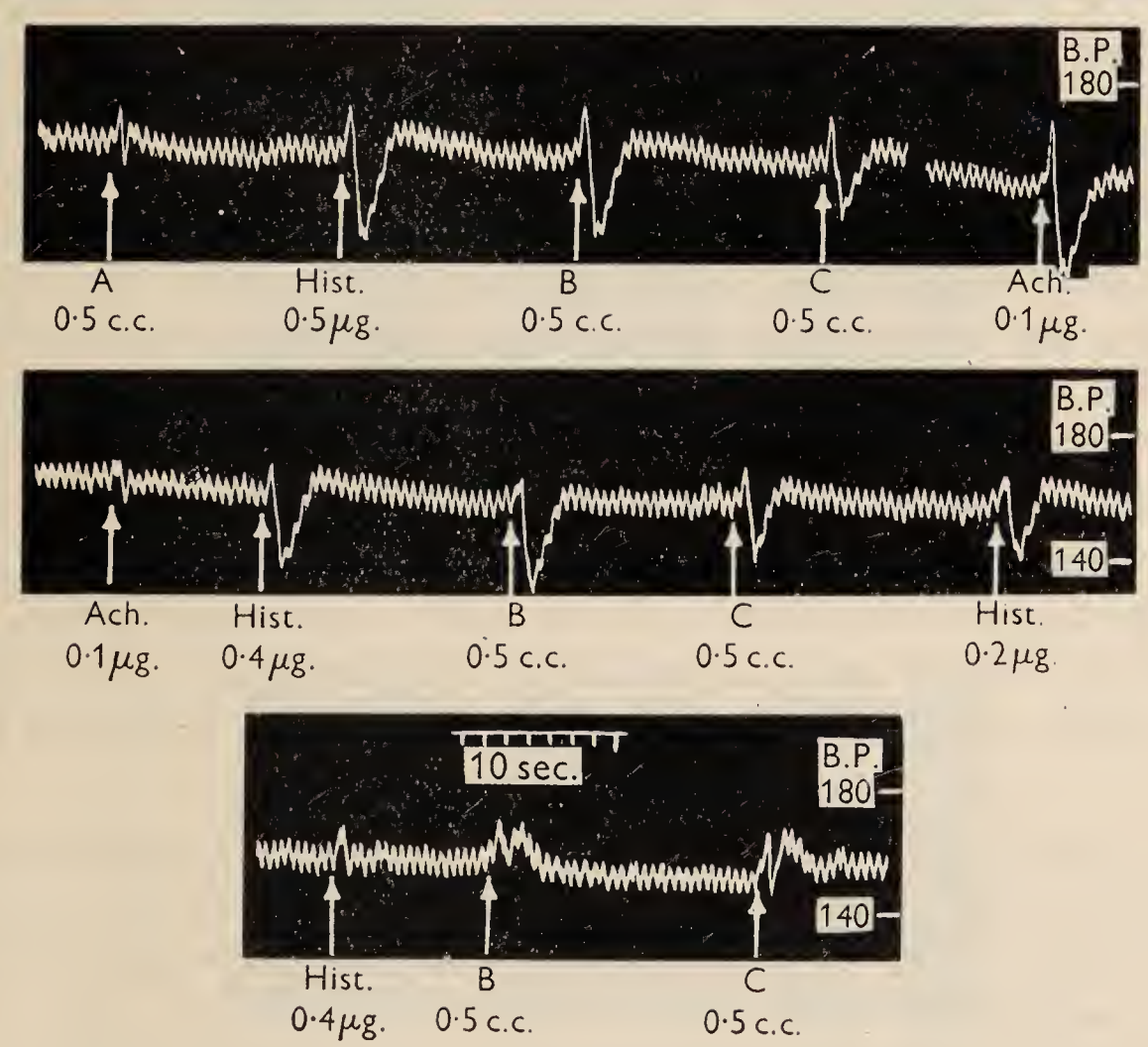


Fig. 6. Cat, chloralose: blood pressure. Injection of heparinized arterial plasma samples from a second cat. A, sample obtained before injection of licheniformin hydrochloride (30 mg./kg.); B, C, samples obtained 1 and 35 min. after the injection. Hist., histamine. Ach., acetylcholine chloride. Between first and second tracings, injection of 0·8 mg./kg. of atropine sulphate; between second and third tracings, injection of 0·8 mg./kg. of Neoantergan maleate.

effects on intravenous injection, and gives rise to inconvenient foaming when tested in the isolated-organ bath. Plasma from cats which had received licheni-

formin produced effects on the blood pressure strikingly like those of histamine, and when the concentration of the depressor material was assayed in terms of histamine at various levels of dosage the same estimate was obtained. The depressor effect of such plasma was unchanged by previous administration of atropine in an amount sufficient to annul the effect of an equidepressor dose of acetylcholine, but it was abolished by an amount of Neoantergan just sufficient to abolish the effect of an equidepressor dose of histamine. Bovet and his co-workers (Bovet, Horclois & Walthert, 1944; Bovet, Horclois & Fournel, 1944) have shown that Neoantergan is a fairly specific antagonist to most of the effects of histamine, including its depressor action. Fig. 6 is the record of a typical experiment.

In addition to its depressor activity, plasma withdrawn from a cat which had received licheniformin produced a contraction of the isolated guinea-pig's ileum, indistinguishable from that produced by histamine. (Licheniformin itself in concentrations as high as 10^{-4} had no effect on the isolated gut, and did not modify its response to histamine.) It was usually possible to express the activity of the plasma in terms of the equivalent concentration of histamine, and the values then obtained agreed with those found in a parallel assay on the cat's blood pressure, within the error of the two methods. In some experiments plasma obtained before the injection of licheniformin either produced a slow contraction of the gut muscle, or modified its sensitivity to histamine, so that the quantitative assay failed; but it was always clear that the injection of licheniformin had greatly increased the histamine-like activity of the plasma. The addition to the bath of atropine sulphate to give a concentration of 4×10^{-7}, which abolished the effect of an equipotent dose of acetylcholine, slightly reduced the response to post-licheniformin plasma, and in about the same degree the response to histamine. Neoantergan (10^{-9}) abolished the response to post-licheniformin plasma and to histamine, but not to other stimulants of smooth muscle (acetylcholine, K^+, Ba^{++}). The pre-treatment of the gut with excess of histamine (Barsoum & Gaddum, 1935) had a similar effect.

Finally, extracts of plasma were made by Code's (1937) modification of the method of Barsoum & Gaddum (1935), involving the destruction through acid hydrolysis of most of the substances acting like histamine on the blood pressure and on the isolated intestine which are likely to be present in tissue extracts. The extracts were assayed against histamine on these two test objects, atropine being present in each case. The results agreed with each other, and with those obtained for the fresh plasma. Fig. 7 and Table 1 give the result of such an experiment.

Thus, soon after the administration of licheniformin to a cat its plasma is found to contain a depressor substance which is not licheniformin itself, but which resembles histamine not only in its depressor effect, but also in its action on

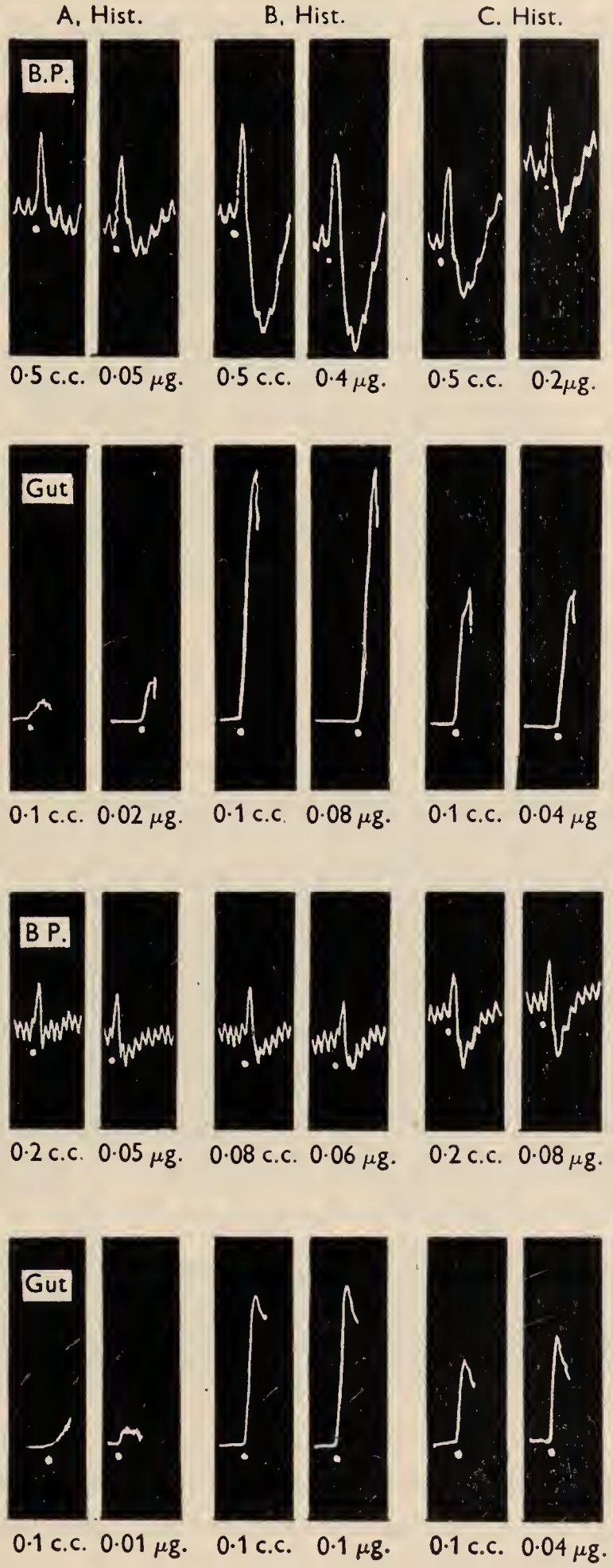


Fig. 7. Assay of samples of heparinized cat's plasma on cat's blood pressure and guinea-pig's intestine. A, plasma obtained before injection of licheniformin hydrochloride 30 mg./kg. B and C, samples obtained 1 and 35 min. after the injection. Hist., histamine. The upper half of the figure compares the effects of histamine and the untreated plasma; the lower half compares the effects of histamine and extracts of the plasma made by Code's method. Volumes are expressed in terms of the original plasma.

intestinal smooth muscle, in its behaviour in the presence of atropine, Neoantergan, and excess of histamine, and in its resistance to acid inactivation. This resemblance, and the quantitative agreement among the results of all the tests in which post-licheniformin plasma was compared with histamine, seem to us sufficient evidence for the conclusion that the depressor substance is histamine itself, and that no other substance contributes in any important

TABLE 1. Histamine equivalent (μg./c.c.) of plasma from a cat treated with licheniformin hydrochloride (30 mg./kg.)

	Before licheniformin	1 min. after licheniformin	35 min. after licheniformin
Fresh plasma, tested on blood pressure	<0·10	0·80	0·40
Fresh plasma, tested on gut	<0·10	0·80	0·42
Plasma extracts, tested on blood pressure	<0·20	0·75	0·40
Plasma extracts, tested on gut	<0·10	0·90	0·35

degree to the depressor activity. As there is no reason to think that the cat can convert licheniformin into histamine, it must be supposed that histamine is discharged from the tissues under the influence of licheniformin. We have some direct evidence that this occurs; in the one experiment of this type that we have made, the histamine content of the skin of a cat's leg fell from 32 to 20 μg./g., after the injection of a large dose of licheniformin.

Experiments with amines, amidines, guanidines and isothioureas

Since licheniformin concentrates gave the Sakaguchi test for guanidine derivatives (Callow *et al.* 1947), we examined a number of bases containing the guanidine group or a related radical. (Unless otherwise noted, compounds were tested in the form of their hydrochlorides or dihydrochlorides.) Some of these were found to act on the chloralosed cat in the same way as licheniformin, the blood pressure beginning to fall steeply some 20 sec. after an intravenous injection, with no immediate change of the heart rate. The active compounds tested varied considerably in effectiveness; some were more and some less active than licheniformin. In a few cases there appeared to be some minor variations in the abruptness and the duration of the blood-pressure fall, but more often the blood-pressure records were indistinguishable from those made with licheniformin. Compounds which produced the delayed depressor effect showed two other phenomena which we have described for licheniformin: the increased response to the second of two small doses, and the large increase of effect with a small increase of dose.

Of the compounds which acted like licheniformin on the blood pressure, the simplest were aliphatic *diamines* homologous with ethylene diamine (Fig. 8). Trimethylene diamine and tetramethylene diamine (putrescine) were inactive. Cadaverine (pentamethylene diamine) lowered the blood pressure, as Barger & Dale (1910) found, but in this case the effect began within a few seconds of the

injection. The C_6 base, however, produced a typical delayed effect; it was about one-quarter as active as licheniformin. Steadily increasing activity was shown by the members of the series with 7, 8, 9, 10 and 11 carbon atoms, the latter being about twice as effective as licheniformin; the C_{12} base was somewhat less

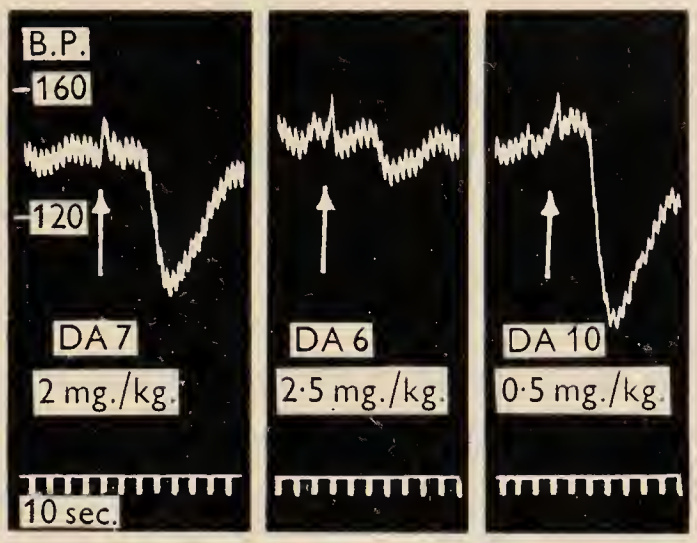


Fig. 8. Cat, chloralose: blood pressure. Delayed depressor responses to polymethylene diamines: doses refer to dihydrochlorides. DA 7, diaminoheptane; DA 6, diaminohexane; DA 10, diaminodecane. 10 min. between injections.

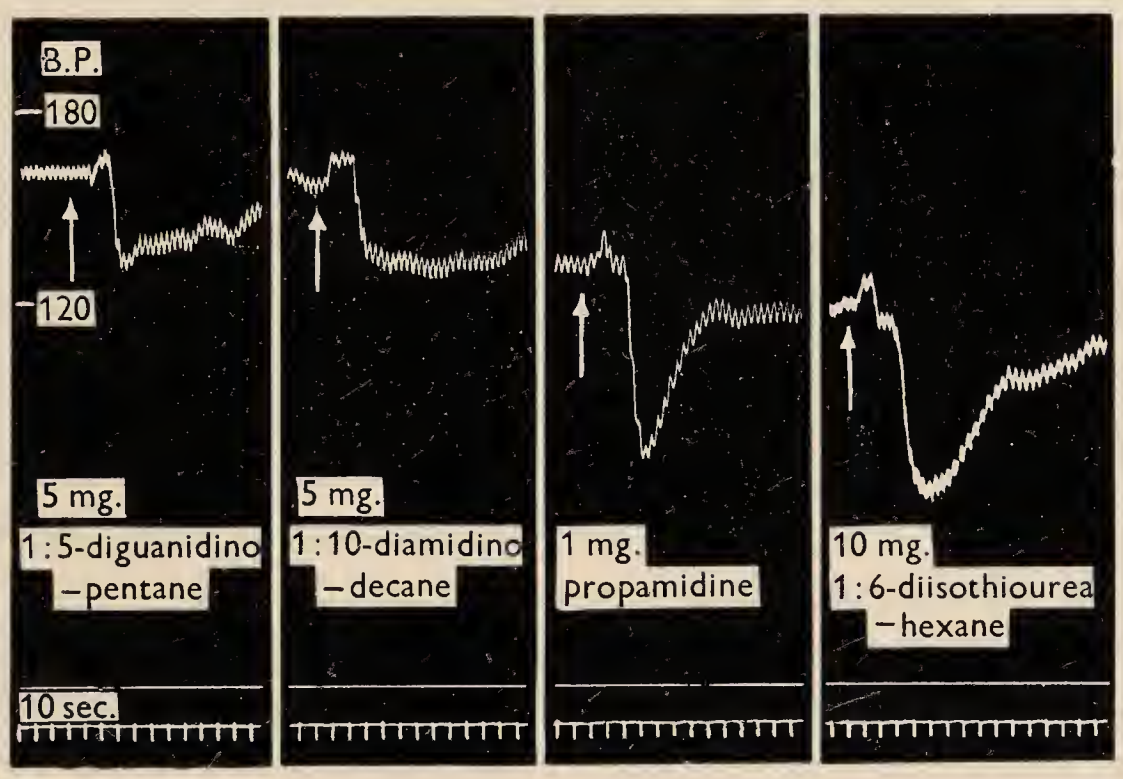


Fig. 9. Cat, chloralose 2·5 kg.: blood pressure. Delayed depressor responses to compounds with basic groups at each end of the molecule. Doses refer to dihydrochlorides.

active. The C_{16} base produced a mixture of pressor and depressor effects, and appears to combine the vasoactive properties of long-chain primary monoamines (Barger & Dale, 1910) with those of the shorter diamines.

Simple aliphatic *diamidines*, *diguanidines* and *diisothioureas*, having the basic groups placed at either end of a carbon chain, also produced the delayed depressor effect (Fig. 9); they appeared to be somewhat more active than the corresponding diamines. Two diquaternary bases, α-ω-*bis*-trimethylammonium

decane and dodecane diiodide, were tested. In the doses used these lower the cat's blood pressure through their action in paralysing sympathetic ganglia; but in the fully nicotinized cat, in which such action is no longer demonstrable, a typical delayed depressor response was obtained. We have also been able, under the same conditions, to obtain a typical effect with D-tubocurarine chloride, as would be expected from the finding of Schild & Gregory (1947) that this compound can liberate histamine. We examined a number of aliphatic primary monoamines, monoamidines, monoguanidines and monoisothioureas; they produced various effects on the blood pressure, but none resembling that of licheniformin. Many compounds of this sort, indeed, are pressor agents (Fastier & Smirk, 1943, 1947).

The ability to produce the delayed depressor effect is not confined to simple aliphatic bases. The most active substances we tested, in fact, were members of the series of trypanocidal aromatic diamidines investigated by King, Lourie & Yorke (1938). Two of these, stilbamidine (4:4-diamidinostilbene) and propamidine (4:4-diamidinodiphenoxypropane; Fig. 9) were about three times as effective as licheniformin. Wien (1943), who described the depressor action of these and related substances, did not refer to its peculiar time relations, but his tracings clearly show the characteristic latent period. We also found a number of *monoamidines* derived from benzamidine which produced the delayed depressor effect; some of these compounds, whose antibacterial and antirickettsial properties have aroused interest, have other pharmacological effects resembling those of the trypanocidal diamidines (Dawes, 1945). In this series, the presence of a polar group remote from the terminal amidine radical appears to be a necessary, though not sufficient, condition for the presence of the characteristic depressor activity. Our tests, however, have been confined to those compounds which were readily available to us, and we have not so far attempted any close study of the relations between chemical structure and activity of the licheniformin type.

Some of the effective compounds, especially those with long aliphatic chains, lower surface tension at an air-water interface when present in high dilution. This surface activity did not appear to run parallel with depressor activity, nor did such highly surface-active substances as saponin, bile salts, soaps or cetyltrimethylammonium bromide produce the delayed depressor effect.

The compounds which have been tested for their ability to produce the characteristic delayed depressor effect are listed in Table 2, and the approximate threshold dose is stated for each compound which clearly produced such an effect. No such activity was detected for the other compounds listed, either because they have little or no power of liberating histamine or because they had effects of a different sort on the blood pressure which would have masked any vasodilatation due to released histamine; in the case of all these compounds, we have stated, with the sign > prefixed, the highest dose tested which certainly

TABLE 2

No.	Compound	Approximate threshold dose (mg./kg.)	Remarks
1	Licheniformin hydrochloride	0·3–1·0	—
	Diamines:		
2	$NH_2(CH_2)_2NH_2$,2HCl	>20	Slight depressor action of short latency
3	$NH_2(CH_2)_3NH_2$, 2HCl	>8	—
4	$NH_2(CH_2)_4NH_2$, 2HCl	>9	Slow depressor action of short latency
5	$NH_2(CH_2)_5NH_2$, 2HCl	>2	Depressor effect of short latency
6	$NH_2(CH_2)_6NH_2$, 2HCl	1·8	—
7	$NH_2(CH_2)_7NH_2$, 2HCl	1·25	—
8	$NH_2(CH_2)_8NH_2$, 2HCl	0·9	—
9	$NH_2(CH_2)_9NH_2$, 2HCl	0·8	—
10	$NH_2(CH_2)_{10}NH_2$, 2HCl	0·5	—
11	$NH_2(CH_2)_{11}NH_2$, 2HCl	0·5	—
12	$NH_2(CH_2)_{12}NH_2$, 2HCl	1·0	—
13	$NH_2(CH_2)_{16}NH_2$, 2HCl	10	Pressor action also
	Diamidines, Am = —C(=NH)NH$_2$:		
14	Am$(CH_2)_8$Am, 2HCl	1·2	—
15	Am$(CH_2)_{16}$Am, 2HCl	1·5	Pressor action also
16	Am—O—$(CH_2)_3$—O—Am, 2HCl (Propamidine)	0·3	
17	Am—CH=CH—Am, 2HCl (Stilbamidine)	0·3	—
18	Am, Am, 2HCl (V 285)	3	—
19	Am—SO_2—Am, 2HCl (V 181)	0·3	—
	Diguanidines, Guan = —NH—C(=NH)NH$_2$:		
20	Guan$(CH_2)_5$Guan, 2HCl	2·5	—
21	Guan$(CH_2)_{10}$Guan, 2HCl (Synthalin)	1	—
22	Guan$(CH_2)_{18}$Guan, 2HCl	7	Pressor action also
	Diisothioureas, ITU = —S—C(=NH)NH$_2$:		
23	ITU$(CH_2)_6$ITU, 2HBr	2·8	—
24	ITU$(CH_2)_{16}$ITU, 2HBr	2	—
	Diquaternaries:		
25	$(CH_3)_3N(CH_2)_6N(CH_3)2I$	>10	All these compounds were tested after paralysis of autonomic ganglia with nicotine tartrate or tetraethylammonium iodide
26	$(CH_3)_3N(CH_2)_{10}N(CH_3)_32I$	>4	
27	$(CH_3)_3N(CH_2)_{12}N(CH_3)_32I$	5	
28	*d*-Tubocurarine chloride	0·6	
29	*o*-Methyl-*d*-tubocurarine iodide	0·6	

TABLE 2 (*cont.*)

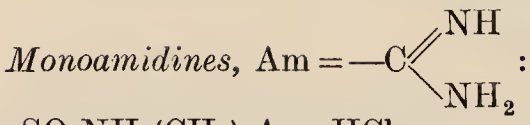
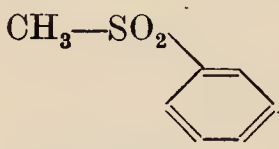
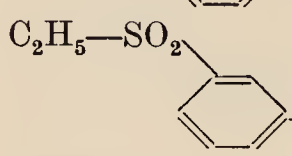
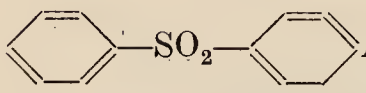
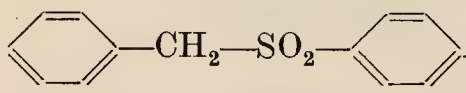
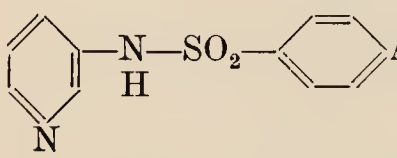
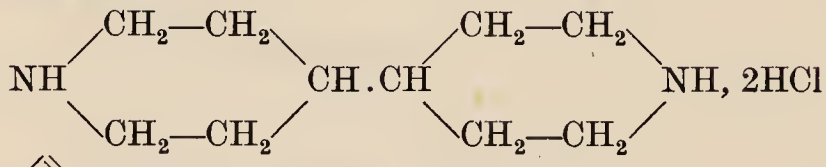
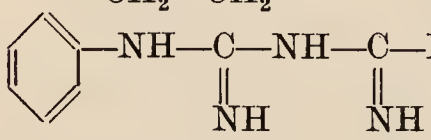
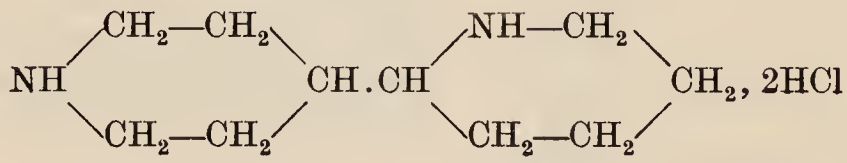
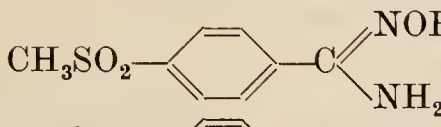
No.	Compound	Approximate threshold dose (mg./kg.)	Remarks
	Monoamidines, Am =  :		
30	$SO_2NH_2(CH_2)_6Am$, HCl	6	—
31	 Am, HCl	>18	Feeble pressor action
32	$CH_3—SO_2$—  Am, HCl (V 187)	5	
33	$C_2H_5—SO_2$—  —Am, HCl	6	—
34	$C_3H_7—SO_2$—  —Am, HCl	4	—
35	 Am, HCl	5	—
36	 Am, HCl	5	—
37	 Am, HCl	1	—
38	 Am, HCl	>3	—
39	$NH_2—SO_2$—  Am, HCl (V 147)	9	—
40	$NH_2—SO_2$—  CH_2—Am, HCl	>17	—
41	 Am, HCl	8	—
42	NH_2—  Am, HCl	6	
43	C_2H_5O—  Am, HCl	6	—
	Miscellaneous:		
44		2·5	—
45		3·5	—
46	$NH_2(CH_2)_4NH.C$(=NH)NH_2	30	—
47		>4	—

TABLE 2 (*cont.*)

No.	Compound	Approximate threshold dose (mg./kg.)	Remarks
48	 , HCl	>13	Cf. no. 32
49	 , HCl	>30	—
50	 , HCl (Marfanil)	>30	—
51	Guanidine carbonate	>18	—
52	Creatinine (Base)	>7	—
53	Nicotinamide	>4	—
54	Aneurine chloride	>40	—
55	Paludrine acetate	>4	Depressor action of short latency
56	Neoarsphenamine	>100	Depressor action of short latency
57	Procaine hydrochloride	>12	Gradual depressor action of short latency
58	Strychnine sulphate	>3	Convulsant
59	Tetramethylammonium iodide	>45	After paralysis of autonomic ganglia with nicotine
60	6-Methoxy-4-quinolylethylamine, HCl	>4	Convulsant
61	6-Methoxy-4-quinolylethylguanidine, HCl	>3	Depressor action of short latency
62	Saponin (B.D.H.)	>0·7	Depressor action of short latency
63	Sodium glycotaurocholate	>4	Depressor action of short latency
64	Cetyltrimethylammonium bromide	>4	—

failed to produce the delayed fall of blood pressure, and the nature of the blood-pressure response where one was observed.

We expected that the compounds listed here as active would act, like licheniformin, by the liberation of histamine. This expectation has been verified for two of them, propamidine and 1:8-diamino-octane, by tests identical with those already described for licheniformin. Table 3 gives the results obtained in the diamino-octane experiment, and Fig. 10 shows part of the pharmacological comparison between histamine and the plasma obtained in the propamidine experiment. Similar tests on dogs with propamidine and another substance, 1:10-diamidinodecane, have given equally clear-cut results. The experiments on dogs will be discussed in detail in a later section.

These results, together with others described below, have made us confident that all the compounds listed as active in Table 2 act on the circulation by the same mechanism. We shall, therefore, refer to them henceforth as 'histamine liberators'.

Although we do not know of any compound, other than those to which we have referred as 'histamine liberators', whose effect on the cat's blood pressure is at all similar to that of licheniformin, we do not wish to suggest that an activity of this type is in itself proof that a compound acts by liberating

histamine. If, however, the compound is closely similar in structure to one of the known liberators, the presumption would be strong that its mode of action is the same.

TABLE 3. Histamine equivalent (μg./c.c.) of plasma from a cat treated with diamino-octane dihydrochloride (13 mg./kg.)

	Before diamino-octane	1 min. after diamino-octane	34 min. after diamino-octane
Fresh plasma, tested on blood pressure	<0·03	0·63	0·16
Fresh plasma, tested on gut	<0·01	0·68	>0·08 <0·20
Plasma extracts, tested on blood pressure	<0·15*	0·70*	0·30*
Plasma extracts, tested on gut	0·02	0·53	0·16

* Depressor activity not wholly abolished by Neoantergan.

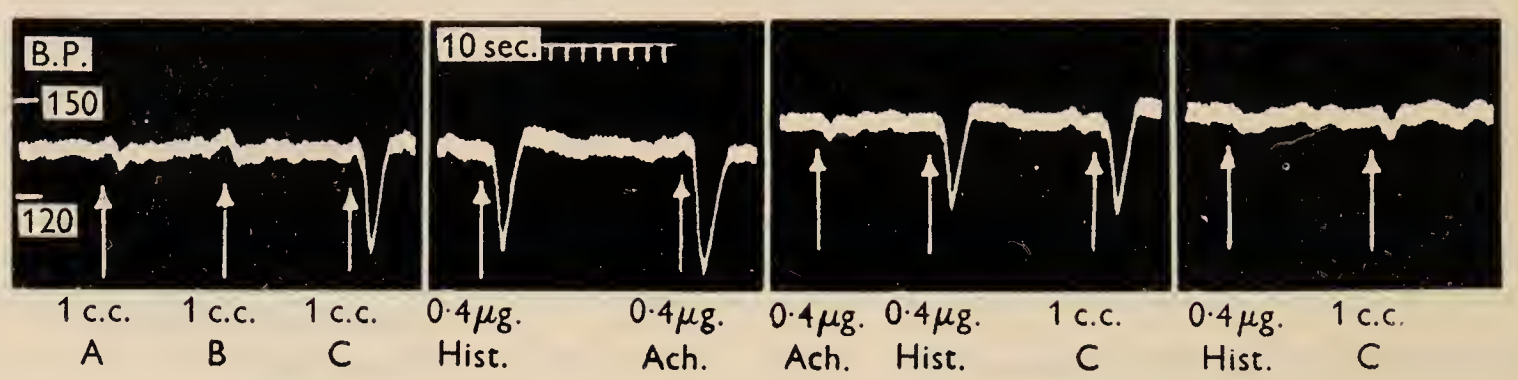


Fig. 10. Cat, chloralose: blood pressure. Effect of histamine and of heparinized plasma from a cat anaesthetized with chloralose. A and B, control plasma samples. C, plasma withdrawn 1 min. after injection of propamidine dihydrochloride 5 mg./kg. Hist., histamine. Ach., acetylcholine chloride. Between the second and third parts of the record the cat received 0·4 mg./kg. of atropine sulphate, and between the third and fourth parts of the record it received 0·4 mg./kg. of Neoantergan maleate.

Other effects of histamine liberators

The experiments so far described have dealt with the effect of histamine liberators on the blood pressure of cats. The compounds of this group have other characteristic effects which appear to depend on the release of histamine.

Intestine. It has already been mentioned that licheniformin has no action on the isolated ileum of the guinea-pig. The same was found to be true of the other histamine liberators we have tested (nos. 8, 14, 16, 20 and 23 of Table 2), when added in concentrations up to 10^{-4} to the Locke's solution surrounding the intestinal strip. As it seemed possible that the alkalinity of Locke's solution might interfere with the action of histamine liberators on the gut, the experiment was repeated with a modified Tyrode's solution adjusted to pH 7·3, but the same negative result was obtained. In concentrations of 10^{-3} or more the guinea-pig's gut responded in some experiments with a brief contraction. Wien (1943) observed that the therapeutic diamidines in similar concentrations contracted the isolated gut of the rabbit. Thus, although the guinea-pig's intestine is known to have a fairly high content of histamine, this is apparently not readily mobilized by these histamine liberators.

On the other hand, we found that some histamine liberators were moderately active in antagonizing the action of histamine on the guinea-pig's ileum. Of the compounds listed above, nos. 16 and 20 had some anti-histamine action in dilutions of 10^{-5} or less, nos. 14 and 23 were effective at the 10^{-4} level, and nos. 1 and 8 had no effect at this dilution. The anti-histamine effect of the compounds was not a very specific one; the action of acetylcholine on the gut was antagonized to about the same extent.

In the anaesthetized cat the injection of various histamine liberators, in doses sufficient to produce a large effect on the blood pressure, caused vigorous contractions of the intestines, visible through the abdominal wall. This is what would be expected in view of the large amount of histamine entering the blood, but reflex stimulation was not excluded.

Gastric secretion. This was measured in a cat anaesthetized with chloralose; the pylorus was tied and the vagi cut in the neck. Warm distilled water in 20 c.c. portions was introduced into the stomach through a catheter passed down the oesophagus, and was withdrawn after 10 min. for the titration of 'free' HCl (methyl red). The slow infusion of a licheniformin hydrochloride solution at a constant rate (0·24 mg./kg. min.) into a femoral vein resulted in an output of free acid, beginning between 10 and 20 min. after the start of the infusion, and outlasting it by about 20 min.; about 85 μM. of acid was secreted. The arterial pressure, which was simultaneously recorded, remained unchanged. The secretory effect would be expected from the ability of licheniformin to liberate histamine; a possible action through disturbance of the CO_2-bicarbonate balance (Browne & Vineberg, 1932) seems unlikely but has not been excluded. Feldberg & Holmes (1941) have made similar observations with 'curarine'.

Haematocrit. As Dale & Laidlaw (1918) showed, a large dose of histamine causes a rise in the haematocrit value. Later work (cf. Stead & Ebert, 1941; Gibson, Seligman, Peacock, Aub, Fine & Evans, 1945; Gibson, Seligman, Peacock, Fine, Aub & Evans, 1946) suggests that such effects as this may be due to the redistribution of blood between large and small vessels as well as to loss of plasma through the capillary wall. Whatever its mechanism, the effect of histamine liberators on the haematocrit value is the same as that of histamine. Fig. 11 shows the effect of a fairly large dose of diamino-octane dihydrochloride (8 mg./kg.) in a chloralosed cat.

Skin. Solutions of drugs for injection into human skin were made in isotonic (1·3%) $NaHCO_3$, so as to be approximately uniform in pH. The injections were made with a fine needle on the flexor surface of the forearm, the volume of fluid being about 0·02 c.c in each instance. The effect was rated in terms of an arbitrary scale, + + + representing a wheal of the maximum size with pseudopodial extensions, + + and + smaller wheals, $\pm$ a doubtful and − a negative effect. The size and intensity of the flare was not taken into account, but appeared to correspond with the degree of whealing. The three subjects

gave very similar responses. The principal results are given in Table 4, and from these the following conclusions can be drawn.

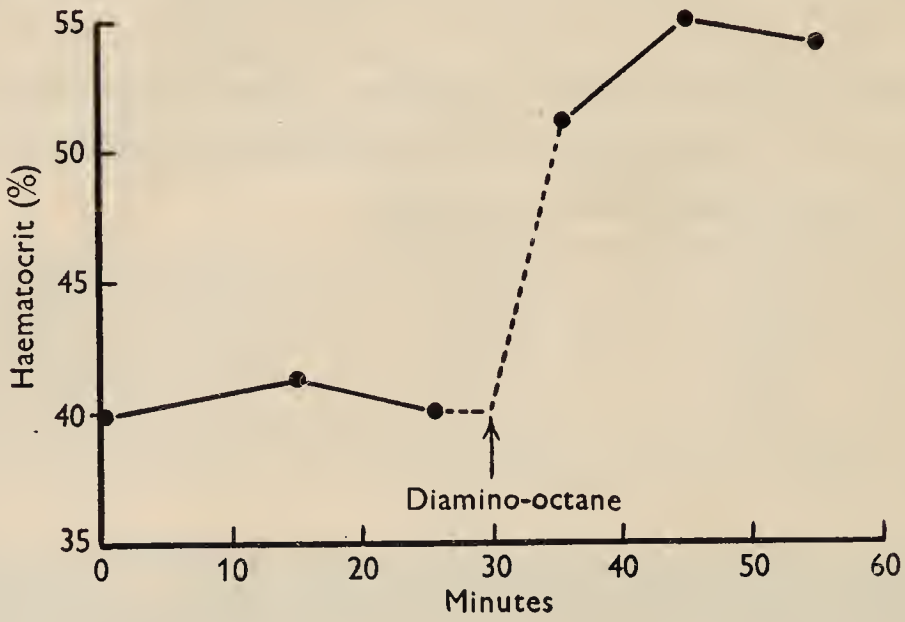


Fig. 11. Effect of the injection of 1:8-diamino-octane dihydrochloride (8 mg./kg.) on the haematocrit value in the cat.

TABLE 4. Whealing of human skin by histamine, histamine liberators and other compounds

No.	Compound	Conc.	Effect: Alone	Effect: With Neoantergan (5×10^{-4})	Effect: On previously injected site
1	Histamine	10^{-4}	+++, +++	...	...
2	,,	10^{-5}	++, ++, +++, ++	...	++, 6 hr. after histamine 10^{-4}; +, 24 hr. after licheniformin 10^{-3}
3	,,	5×10^{-6}	++, ++, +, ++	−, ±, ±	+, 24 hr. after licheniformin 10^{-4}; ++, 24 hr. after diamidinodecane
4	,,	10^{-6}	+, +	...	...
5	,,	10^{-7}	+	...	...
6	,,	10^{-8}	−	...	...
7	Licheniformin	10^{-2}	+++, +++	...	...
8	,,	10^{-3}	+++, +++, +++, +++, +++	...	...
9	,,	5×10^{-4}	++, +++	±, +	±, 24 hr. after licheniformin 10^{-3}
10	,,	10^{-4}	++, +++	...	++, 6 hr. after histamine 10^{-4}; −, 6 hr. after licheniformin 10^{-3}
11	,,	10^{-5}	+, +	...	−, 24 hr. after licheniformin 10^{-3}
12	,,	10^{-6}	+	...	...
13	Diamino-octane	10^{-2}	+++, +++	...	...
14	,,	10^{-3}	++, ++, ++	...	...
15	,,	5×10^{-4}	++, +	±	±, 24 hr. after diamino-octane 10^{-}
16	Diamidinodecane	10^{-3}	++, +++, ++	...	...
17	Diguanidinopentane	10^{-2}	++, ++	...	...
18	,,	10^{-3}	++, +, ++	...	...
19	Diisothioureahexane	10^{-3}	++, ++	...	...
20	V 187	10^{-3}	++	...	...
21	Putrescine	10^{-3}	±	...	...
22	Benzamidine	10^{-3}	−, ±, ±	...	...
23	Neoantergan	10^{-3}	−, ±, −	...	...

Concentrations of histamine refer to the base; those of licheniformin, V 187 and benzamidine to the hydrochloride; t of Neoantergan to the maleate; those of the other compounds to the dihydrochloride.

(1) Compounds which were active histamine liberators, as judged from their effectiveness in the test on the cat's blood pressure, all produced a typical wheal-and-flare response identical with that evoked by histamine, when they were injected intradermally in high dilution (nos. 7–20). No persistent in-

flammation or necrosis was caused by any of the compounds in the concentrations used.

(2) Conversely, no wheal or flare was produced by related compounds (nos. 21, 22) showing no activity in the blood-pressure test.

(3) Neoantergan added to a histamine solution greatly reduced or abolished its effects on the skin (no. 3); it also counteracted the effects of histamine liberators (nos. 9, 15). Dews & Graham (1946) have shown that Neoantergan prevents the development of histamine wheals in human skin, and Parrot (1942) has found that the related base Antergan inhibits the wheal-and-flare response to stroking the skin, which is mediated, as Lewis (1927) demonstrated, by the liberation of histamine or a similar substance. It must be remembered, however, that local anaesthesia also reduces the wheal and abolishes the flare, produced by histamine (Lewis & Grant, 1924). Since Neoantergan is a powerful local anaesthetic (Dews & Graham, 1946), its inhibition of the cutaneous effects of histamine liberators may depend on this property rather than on its specific anti-histamine properties.

(4) An area of skin which has been the site of a wheal produced by a histamine liberator is refractory, for 24 hr. at least, to the production of a second wheal by the same agent (nos. 9, 10, 11, 15), although histamine is still effective (nos. 2, 3). Either histamine or a histamine liberator will produce a second wheal on the site of a histamine wheal (nos. 2, 10). A possible explanation, which we have not tried to verify, is that the local store of histamine remains depleted for a long time after the injection of a histamine liberator. A similar phenomenon was described by Grant, Pearson & Comeau (1935), who noted that skin areas in which whealing had been produced by warming or by a parasympathomimetic drug would not again form wheals in response to the same stimulus, unless an interval of about 2 days had elapsed.

It is clear that all these effects are most easily explained on the assumption that the compounds in question liberate histamine when introduced into the skin. If it be supposed that histamine, locally liberated in this way, has the same effect weight for weight as injected histamine, the amounts of histamine released can be roughly calculated. On this basis, for example, between 10 and 100 molecules of diamidinodecane are required to liberate a molecule of histamine.

Miles (1948) has observed that the intradermal injection of licheniformin, stilbamidine or V187 into a guinea-pig which had received pontamine blue intravenously is followed by a local blue discoloration of the skin, similar to that produced by an intradermal injection of histamine.

Blood pressure of the dog. Most of our experiments on the vascular effects of histamine liberators were done on cats. Dogs were used in a number of later experiments, the results of which differed somewhat from those observed in cats, and will therefore be described separately.

Each of the histamine liberators tested produced a sharp fall of blood pressure when given by vein to anaesthetized dogs, and in some animals the fall occurred after an interval of 20–25 sec., just as in the cat anaesthetized with chloralose. More often, however, it began within 10 sec. of the injection, and in that case it was sometimes possible to note a second phase of the depressor effect beginning some 15 sec. later. In one dog, the depressor response could be changed repeatedly from an immediate to a delayed one by the continuous infusion of adrenaline at a rate (6 μg./kg. min.) barely sufficient to elevate the blood pressure. The latency of the blood-pressure fall produced by diamino-octane was raised from 8 ± 1 sec. in the absence of adrenaline to 24 ± 3 sec. during the infusion, whereas the latency of the fall produced by histamine was constantly

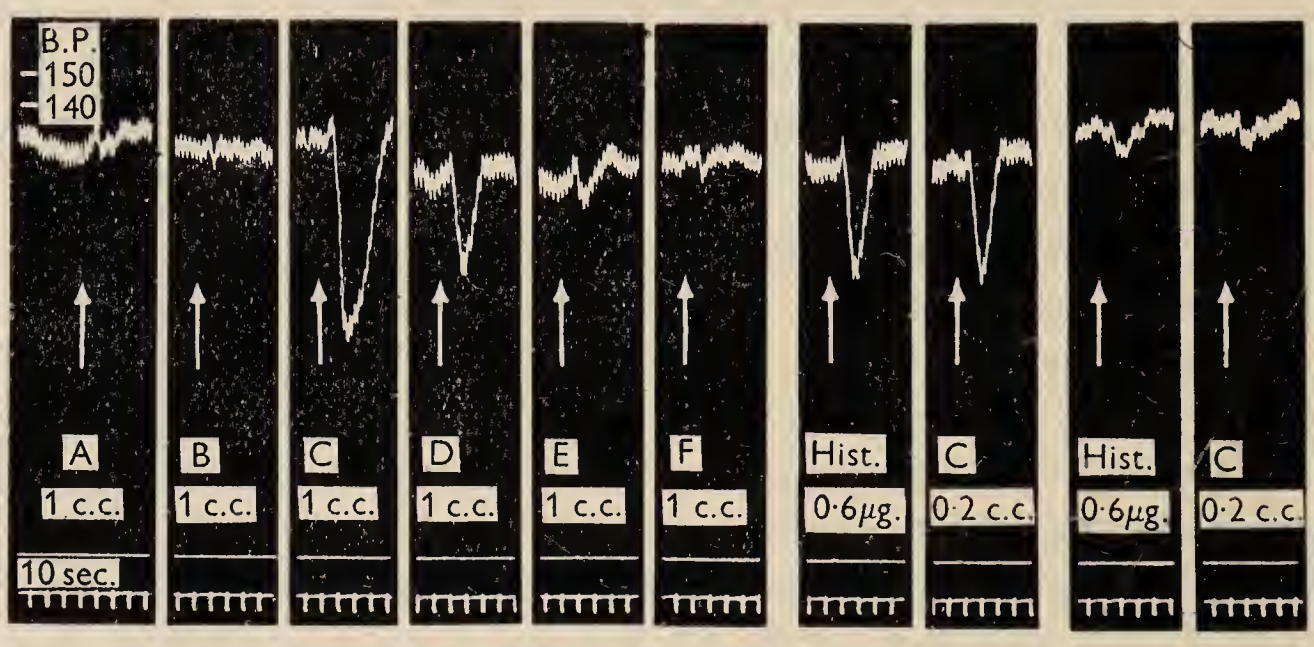


Fig. 12. Cat, chloralose: blood pressure. Effect of histamine and of heparinized plasma from a dog anaesthetized with chloralose-urethane. Hist., histamine. A to F, plasma samples; A and B, 20 and 10 min. before injection of diamino-octane (see text); C, D, E and F, 1, 13, 31 and 106 min. after the injection. Before the last two injections the cat received 0·3 mg./kg. of Neoantergan maleate.

5–6 sec. Individual dogs varied more than individual cats in their sensitivity to histamine liberators, but on the average were equally or slightly more sensitive: the nature and size of the depressor effect were not obviously related to the anaesthetic used. The enhanced response to the second of two closely succeeding injections of a histamine liberator, which has been described above for the cat, was not seen in the dog.

Dog's plasma obtained after the injection of large doses of a histamine liberator contained histamine: the methods used for its identification and assay were those already described for histamine in cat's plasma. The observed increases in plasma histamine were even larger than those found in the cat. Thus the following values were observed in different dogs 1 min. after the injection of a liberator: 3·0 μg./c.c. after diamino-octane dihydrochloride (15 mg./kg.); and 2·0 μg./c.c. after diamidinodecane dihydrochloride (15 mg./kg.). Fig. 12

shows the depressor effects produced by successive plasma samples in the diamino-octane experiment.

When a histamine liberator was injected into a dog with opened abdomen, the liver could be seen to darken and to swell dramatically as the blood pressure fell, and to resume its normal appearance as the blood pressure recovered.

In a few dogs, for reasons which are unknown, the usual effects of histamine liberators on the blood pressure or on the plasma histamine level were either much reduced, or absent, or could be elicited only at the outset of the experiment.

Blood coagulation. We have never observed any retardation in the clotting of the blood of cats as the result of the administration of histamine liberators even in doses large enough to produce shock. In dogs, on the other hand, the

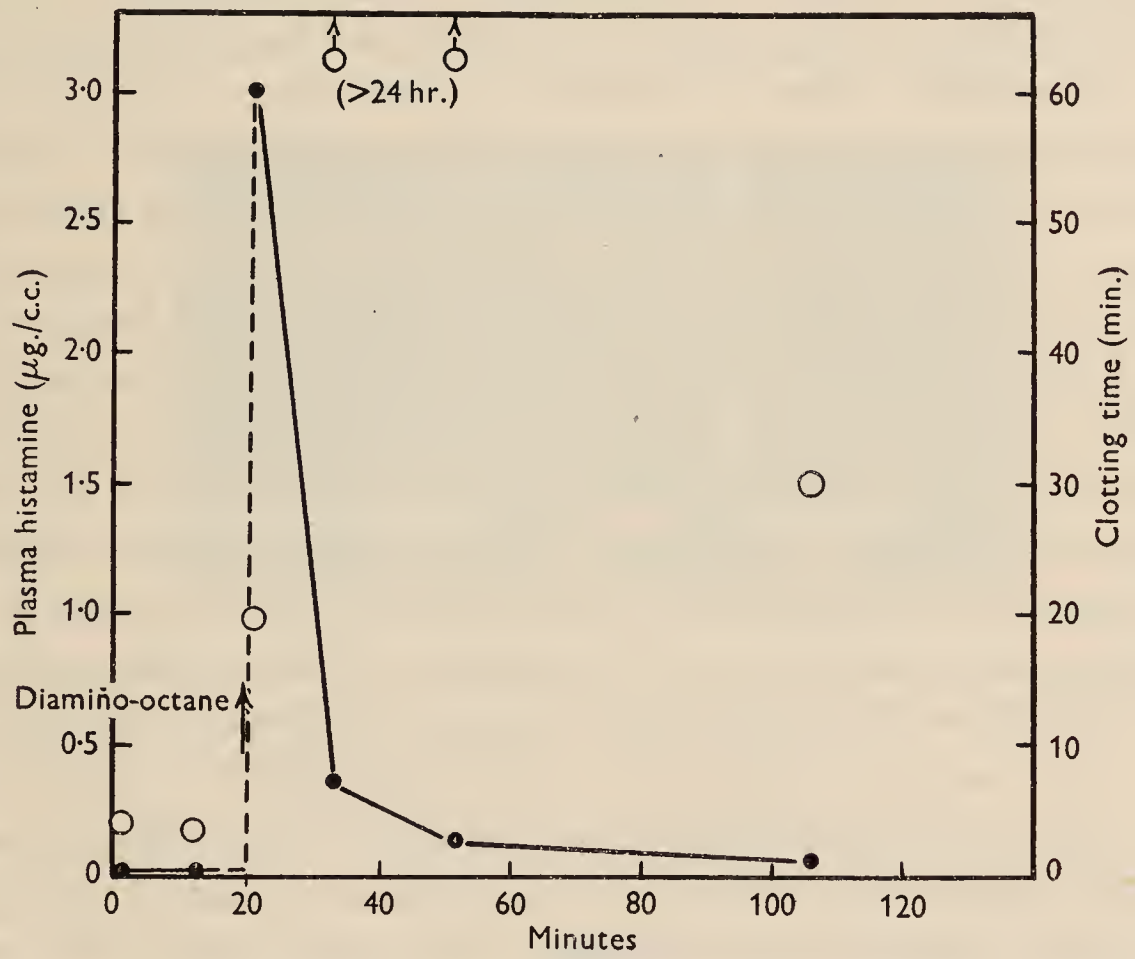


Fig. 13. Dog. Same experiment as Fig. 12. Effect of diamino-octane dihydrochloride (15 mg./kg.) on clotting time of arterial blood (circles) and on plasma histamine content (dots).

clotting time of arterial blood has shown a striking increase in every experiment in which a release of histamine was observed. The data of one such experiment are shown in Fig. 13. It will be observed that the effect on clotting in this experiment did not become maximal until the plasma histamine had begun to fall, and this result was also obtained in the other experiments. Histamine liberators added to dog's blood *in vitro* had no effect on the clotting time. Histamine itself affects the clotting time very little, either *in vivo* or *in vitro*.

The effects of histamine liberators on the dog appeared to be similar to those observed in peptone and anaphylactic shock, in which the incoagulability of

the blood is due to the presence of heparin (Wilander, 1938; Jaques & Waters, 1940). The clotting defect provoked by histamine liberators is apparently caused in the same way. Toluidine blue is known to form with heparin a complex in which the dye assumes its metachromatic colour; the heparin so combined has no anticoagulant activity (Jorpes, Holmgren & Wilander, 1937). A sample of incoagulable blood from a dog which had received diamino-octane clotted within a few minutes after the addition of toluidine blue (0·075 mg./c.c. of blood), and plasma from such blood displaced the absorption spectrum of toluidine blue toward the violet in the same way as plasma to which heparin had been added. The evidence so far obtained thus suggests very strongly that histamine liberators in the dog are also heparin liberators; but final proof that this is so must await the isolation of heparin from the blood of dogs poisoned by such compounds.

The site of histamine release. We have emphasized that in the chloralosed cat there is no immediate effect of histamine liberators on the blood pressure, although there may be an abrupt and substantial fall after a characteristic latent period of 20–25 sec. This in turn implies either that the histamine is released into a small portion of the vascular bed, or that it enters the blood stream beyond the site of the main capillary resistance, or both of these. In some circumstances, however, the immediate depressor effect is more prominent; this is usually the case in the dog, and in the etherized or decerebrate cat, although these are not necessarily more sensitive to intravenous histamine than the cat anaesthetized with chloralose. An immediate vasodilator effect can also be demonstrated for the diamidines in the perfused limb of the cat (Wien, 1943), provided that the vascular tone is maintained with adrenaline. Possibly the presence or absence of an immediate depressor effect in the intact animal depends on the relative distribution of the peripheral resistance among the arterioles, meta-arterioles (Chambers & Zweifach, 1944) and capillaries. The modification by infused adrenaline of the depressor response in the dog, already referred to, can probably he explained in this way. As to the nature and location of the vessels into which the liberated histamine diffuses, our experiments give no suggestion.

More information is available as to the organs chiefly concerned in the liberation of histamine in the cat. We injected a small dose of licheniformin alternately into the left and right auricle of a cat whose chest had been opened under artificial respiration (Fig. 3). The depressor effect produced by injection into the left auricle was greater, and a few seconds earlier in onset, than that produced by injection into the right auricle. The *lungs*, therefore, do not contribute any large part of the histamine entering the blood (unless, as seems improbable, the histamine is liberated only in those parts of the lungs supplied by the bronchial arteries). The *liver* and *digestive tract* in the cat are also unimportant. Licheniformin injected into the coeliac or hepatic artery, or into the

portal vein, had little effect on the blood pressure unless the dose was large. On the other hand, after evisceration a smaller dose of a liberator was needed to produce a depressor effect than before.

The injection of licheniformin into the central stump of the cut inferior mesenteric artery of an eviscerated cat, so that the drug reached mainly the skin and muscle of the hindlimbs, produced a fall of blood pressure larger than the same dose intravenously. This suggests that most of the histamine liberated in the cat comes from *skin* or *muscle*. We have little information about the relative importance of these two tissues. In the one experiment in which their histamine content was measured, that of the skin fell by 40%, but there was no detectable change in that of the muscle. On the other hand, Wien (1943) observed that the intra-arterial injection of diamidines into a skinned limb was followed by a large fall of blood pressure, and we were able to produce a typical depressor effect in a cat which had been skinned as completely as possible.

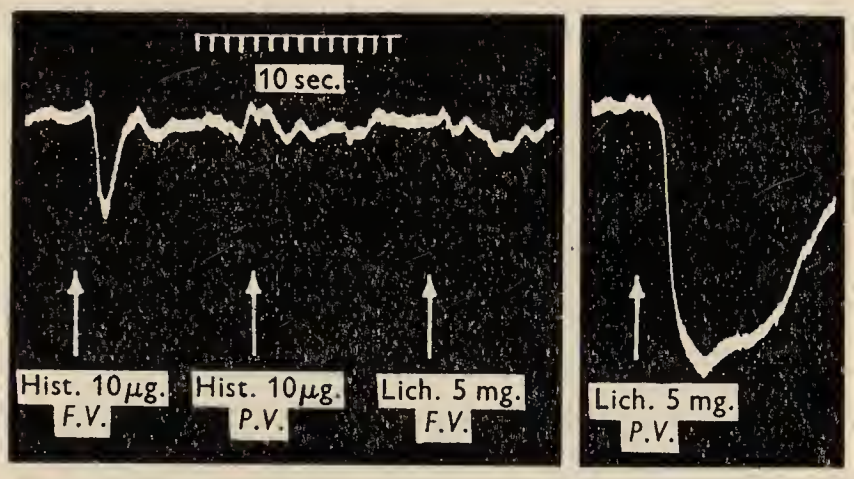


Fig. 14. Dog, sodium barbitone: blood pressure. Effect of injection of histamine (Hist.) and of licheniformin hydrochloride (Lich.) into the femoral vein (*F.V.*) and the portal vein (*P.V.*). The second dose of licheniformin was given 10 min. after the first.

Most of the experiments described above were repeated on the dog. Injection of a histamine liberator into the right auricle was no more effective than injection into the left auricle; and evisceration of the animal left the depressor response unchanged in character, though much reduced in size. These results correspond to those obtained in the cat, and suggest that in the dog the compounds under discussion can liberate histamine from skin and/or muscle, but not to any great extent from the lungs. We did, however, find one striking difference between the two species. In the cat, the liver does not appear to participate in the release of histamine, but in the dog it is the organ chiefly responsible. The evidence for histamine release by the dog's liver comes from two observations: (*a*) as has just been mentioned, the depressor effect of histamine liberators was much smaller after evisceration, although the depressor effect of histamine itself was not reduced; (*b*) injection of a histamine liberator into the portal vein of a dog produced a much greater effect on the blood pressure than injection of the same dose into a systemic vein, whereas histamine itself

was less effective by the intraportal route (Fig. 14). These results were the opposite of those obtained in the cat, which became more sensitive to histamine liberators after evisceration and gave a bigger response to an ordinary intravenous injection than to an intraportal one.

Pharmacological similarity to peptone. In both the cat and the dog, the effects of histamine liberators closely imitate those of peptone, and, almost as closely, those of antigens in sensitized animals. Peptone, like histamine liberators but unlike histamine, lowers the dog's blood pressure more when given intraportally than when given intrajugularly (Feldberg, Schilf & Zernik, 1928), and is more effective in the intact than in the eviscerated dog (Feldberg *et al.* 1928; Mautner & Pick, 1929). The toxic effects of antigens in sensitized dogs are likewise well known to depend on the presence of the liver. Both these agents cause a reduction in the histamine content of the liver which apparently is sufficient to account for the concomitant increase in the histamine content of the body fluids (Ojers, Holmes & Dragstedt, 1941; Holmes, Ojers & Dragstedt, 1941). No doubt the compounds we have examined would similarly reduce the stored histamine of the dog's liver, but we have made no estimations of liver histamine.

In the cat, on the other hand, the liver plays no active role in the production of peptone shock (Feldberg, 1929) or of anaphylaxis (Edmunds, 1914); and we have found it equally unimportant in determining the action of histamine liberators.

It is well known that a dog which has recovered from peptone shock is little affected by a second injection of peptone. In the one experiment of this sort which we did, we found that the induced refractoriness to peptone also extended to the effects of a histamine liberator. A dog anaesthetized with chloralose-urethane received 400 mg./kg. of 'Difco proteose-peptone' from which the free histamine had been removed by treatment with Permutit. The dog went into shock; the plasma histamine level rose to 0·42 μg./c.c., and the blood became completely incoagulable. After 75 min., the plasma histamine had fallen below 0·03 μg./c.c. and the clotting power of the blood had returned to normal. A large dose of diamidinodecane dihydrochloride (10 mg./kg.) was then injected: it produced only a trivial rise of plasma histamine, to 0·05 μg./c.c., and no effect on the clotting time. Many previous workers have found that not only is a refractory state produced by an effective injection of peptone, but that a certain proportion of dogs are from the beginning insensitive to peptone. We have found this to be the case with histamine liberators as well.

A further similarity between the effects of peptone and those of histamine liberators has already been described: this is the incoagulability of the blood which both produce, and which we have tentatively ascribed to the liberation of heparin. We have not been able to produce this phenomenon in the cat.

Lastly, we have noted that in the dog histamine liberators, especially when injected into the portal vein, cause a rise in intraportal pressure and a great increase in the size of the liver. These effects, which depend on constriction of the hepatic veins, are produced by peptone, but also by histamine itself (Feldberg *et al.* 1928; Mautner & Pick, 1929) when given in large doses. Our impression is that the obstruction of the hepatic circulation produced by a histamine liberator is greater, in proportion to the fall of arterial pressure, than that produced by intravenous histamine, and so is to be ascribed, like the similar effect of peptone, mainly to the histamine set free within the liver itself; but we have no quantitative data on this point.

Substances modifying the action of histamine liberators

Anti-histamine drugs. It is somewhat difficult to predict how far these substances would suppress the vascular action of histamine liberators, even though this action is considered to be due solely to the histamine liberated. Neoantergan, Benadryl, and related compounds have only a limited ability

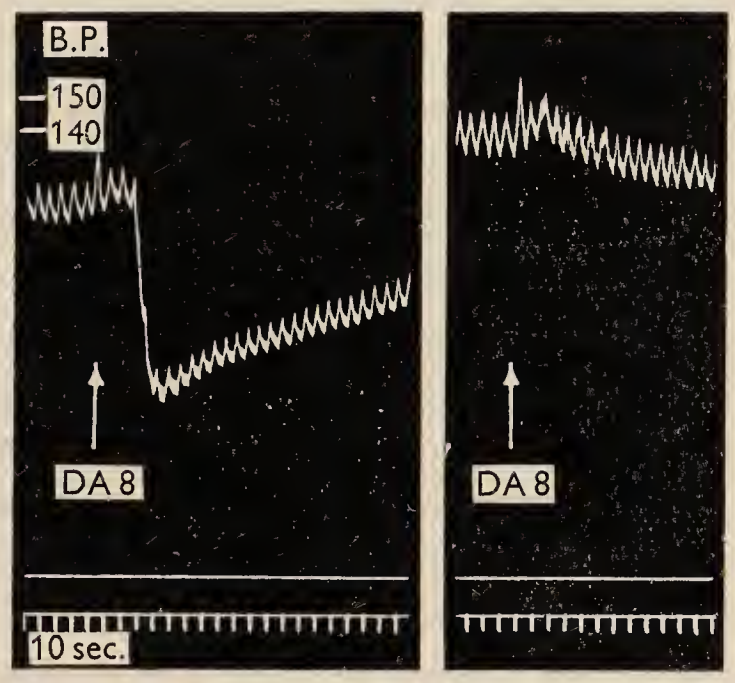


Fig. 15. Cat, chloralose, eviscerated: blood pressure. Effect of diamino-octane dihydrochloride (0·8 mg./kg.) before and after Neoantergan maleate (4 mg./kg.).

to antagonize the depressor action of histamine in the cat and dog (Bovet & Walthert, 1944; Dews & Graham, 1946; Marsh & Davis, 1947). Moreover, the amount of histamine entering the blood when a dose of liberator is injected will generally be much greater than the amount of histamine required to produce an equal reduction in blood pressure when injected suddenly into a vein, as is shown by the greater duration of the effect in the former case. The extent to which histamine can lower the blood pressure depends not only on the plasma histamine concentration, but on the rate at which this is changing (cf. Emmelin, 1946). On the basis of such considerations it might be expected that the anti-histamine drugs would antagonize the circulatory effects of the histamine

liberators less completely than those of histamine. Our experiments indicate that this is true to some extent, but we found, nevertheless, that Neoantergan abolished the depressor effect of a small dose of a liberator (Fig. 15), and retarded the rate at which the blood pressure fell when a larger dose of liberator was given. Further, when the blood pressure had been lowered by repeated injections of a liberator, it was partially restored by Neoantergan or Benadryl, which had no pressor action in the normal animal. Thus, anti-histamine drugs can antagonize the circulatory effects of histamine liberators, but the antagonism is a limited one.

Calcium salts. Wien (1943) observed that the injection of a solution of $CaCl_2$ (10–15 mg.) or calcium gluconate (50–100 mg.) reduced or abolished the depressor action of stilbamidine and propamidine in the cat. We tested the effect of these salts on the vasodilator action of licheniformin and propamidine: in the chloralosed cat, the inhibitory effect was usually small, and consisted mainly in slowing the rate at which the blood pressure fell after a dose of the histamine-liberator; but in the etherized cat calcium appeared to reduce the depressor action considerably.

Heparin. The effect of heparin on the vascular response to histamine liberators was examined because it was noted that some of the compounds form insoluble complexes with heparin, and because heparin is known to inhibit the release by peptone of histamine from rabbit blood cells (Dragstedt, Wells & Rocha e Silva, 1942), and probably also from dog liver (Rocha e Silva, Scroggie, Fidler & Jaques, 1947). In two cats we determined the effect of a large dose of heparin (40–60 mg./kg.) on the depressor response to histamine and to licheniformin: the former was unaffected; the latter was slowed and reduced in size.

Alkalis. In two experiments it was found that doses of licheniformin or diamidinodecane which produced a good depressor effect when dissolved in 1 c.c. of 0·9% NaCl, were nearly or quite inactive when dissolved in 1 c.c. of M/6 NaOH. The alkaline solutions recovered their activity on being neutralized. The phenomenon has not been studied further. The inhibitory effect of heparin and of calcium salts on the depressor response to histamine liberators is not a pH effect.

Splenin. Ungar (1945) has isolated from spleen a substance, apparently a polypeptide, which inhibits, in minute concentrations, the release of histamine in anaphylaxis and peptone shock. Through the courtesy of Dr Ungar we were able to test the effect of this substance, splenin, on the depressor action of licheniformin. Splenin was given intravenously in doses of 6–10 μg./kg. 5–20 min. before the injection of licheniformin, these doses being many times greater than that which Ungar found necessary to inhibit the release of histamine in guinea-pig anaphylaxis. The depressor response to the histamine liberator was unaltered.

Other substances. Raiman, Later & Necheles (1947) have reported that *rutin*, a flavone glucoside, injected intraperitoneally into sensitized guinea-pigs

protects them against anaphylaxis; and its aglycone, *hesperidin*, is said to protect against anaphylactic shock (Hiramatsu, 1941). Each of these compounds, injected intravenously as the sodium salt in doses up to 50 mg./kg., failed to modify the depressor activity of histamine liberators in the cat.

Procaine hydrochloride (15 mg./kg.), putrescine dihydrochloride (12 mg./kg.), and a number of inactive monoamidines all failed to inhibit the response to subsequent injection of a histamine liberator.

The amount of histamine released

The question naturally arises whether the amount of histamine released when a histamine liberator is injected is sufficient to account for the effect of the latter on the blood vessels. We were unable with the methods available to detect any increase in plasma histamine associated with small depressor responses to the injection of either histamine or a histamine liberator. The data in the literature do not suggest any very close correlation between observed values for plasma histamine and the circulatory state of the animal: it is clear, indeed, that the vessels gradually become unresponsive to histamine while the plasma histamine remains at a high level, or even continues to rise (Emmelin, Kahlson & Wicksell, 1941; Emmelin, 1946). In two experiments on cats, we observed that a single intravenous injection of 1 mg./kg. of histamine, which caused a profound but not fatal lowering of the blood pressure, the plasma histamine level 10 min. after the injection was 0·5 μg./c.c.; and Code (1939) found in the dog that the histamine content of venous blood rose to 0·2–0·4 μg./c.c. 1 min. after an injection of 0·3–0·4 mg./kg. of histamine, which produced a similar effect on the blood pressure. It is of interest to note that the values obtained for histamine in dog's plasma during anaphylaxis and peptone shock (Code, 1939; Dragstedt, Mead & Eyer, 1938; Rocha e Silva & Texeira, 1946) are of the same order of magnitude, ranging from 0·2 to about 2 μg./c.c. according to the severity of the shock. In our experiments, the plasma histamine frequently reached very high levels—up to 0·7 μg./c.c. in the cat, and up to 3 μg./c.c. in the dog—when histamine liberators were injected in large doses. There seemed, therefore, good reason to believe that these animals, whose circulatory state was indistinguishable from that of animals poisoned by histamine, were suffering from histamine overdosage. In one or two experiments, however, in which we estimated the histamine in the plasma of cats which had received large doses of histamine, the blood pressure was not lowered so far nor for so long as was usual following an injection of a histamine liberator which caused an equal increase in plasma histamine. Such a discrepancy, if real, might have been due to differences in the distribution of histamine in the two cases, the same level of plasma histamine being associated with different levels of free tissue histamine in the two kinds of experiment. Alternatively, it is possible that histamine liberators do not merely liberate histamine but damage the blood vessels in some other way.

We have found, however, no evidence of any circulating depressor substance other than histamine itself. It seems clear, therefore, that a great part at least of the vascular effects of histamine liberators is in fact due to the histamine set free by them.

DISCUSSION

Our experiments show that many basic organic compounds are able, when present in low concentrations, to liberate histamine from mammalian tissues, and that some of the more striking pharmacological effects of such compounds can be ascribed to the histamine that they liberate. The chemical structure necessary and sufficient to confer histamine-releasing activity cannot as yet be stated precisely. So far the compounds found to be active can be divided into two classes: those in which two basic radicals are separated by a substantial inert moiety of predominantly hydrocarbon nature; and a series of benzamidine derivatives possessing a second polar group remote from the amidine radical.

Many compounds of these two classes have been synthesized and tested for chemotherapeutic activity (cf. King *et al.* 1938; Fuller, 1942; Andrewes, King, van den Ende & Walker, 1944). Of these, at least the trypanocidal diamidines (stilbamidine, propamidine, pentamidine) appear to liberate considerable quantities of histamine when they are given to man by vein in the usual dosage. Some of the transient side-actions of these drugs, including itching, colic, and fall of blood pressure, which have been noted by many observers (Adams, 1941; Saunders, 1941; Lawson, 1942; Lourie, 1942; Kirk & Henry, 1944), may be attributed with confidence to the histamine that is set free. It is desirable that potential therapeutic agents, whose chemical structure suggests that they might liberate histamine, should, before they receive clinical trial be tested on animals like the cat or dog whose blood vessels are sensitive to histamine. Such compounds may, of course, have other toxic effects, not obviously related to the release of histamine: thus, prolonged administration of diamidines causes in animals severe damage to the liver and kidneys (Broom, 1936; Devine, 1940; Wien, Freeman & Scotcher, 1943); the same is true for diguanidines (Blatherwick, Sahyun & Hill, 1927; Bischoff, Sahyun & Long, 1929). Whether this sort of toxicity is dependent on the same types of chemical structure responsible for histamine-releasing activity has yet to be determined.

Our attempts to discover methods of counteracting the effects that we have ascribed to histamine liberation have not been very successful. The anti-histamine substances now available are remarkably active in preventing many effects of injected histamine, especially those on smooth muscle; but they seem, for reasons we have discussed above, to be less efficient in preventing the vascular effects of histamine liberators. Of the substances which appear to modify the release of histamine, rather than its action, heparin possibly acts by forming complexes of low solubility with the liberators, but the action of calcium salts and alkalis cannot be explained in this way. Heparin is known to have

some inhibitory effect on anaphylaxis and on the similar phenomena produced by peptone, both in the whole animal and in isolated tissues (Kyes & Strauser, 1926; Williams & van de Carr, 1927; Dragstedt *et al.* 1942), and calcium has been reported to act in a similar manner (Schittenhelm, Erhardt & Warnat, 1928; Kallós & Kallós-Deffner, 1935).

The release of histamine in our experiments is most simply explained on the supposition that histamine can be displaced by chemically similar bases from its combination with tissue substances. The histamine-liberating effects of diamines and diamidines would be easy to understand on this view, since histamine itself may be regarded as a diamine or an amidine in structure; and the biochemical resemblance of such compounds to histamine is illustrated by the fact that diamine oxidase destroys histamine (Zeller, 1938*a*) and is strongly inhibited by diamidines (Blaschko & Duthie, 1944) and diguanidines (Zeller, 1938*b*). This displacement hypothesis, however, cannot readily explain the ability to release heparin in the dog, which seems to be a characteristic property of histamine liberators: for heparin is not a base, nor is it liberated by histamine. It could perhaps be supposed that histamine and heparin are associated in some tissue complex, and that the displacement of the former mobilizes the latter as well, but we know of no evidence for such an association.

In the dog, as is well known, anaphylactic and peptone shock are also accompanied by the liberation of both histamine and heparin. It is attractive to suppose that a similar mechanism is responsible for the shock produced by histamine liberators. We have made two observations which support this supposition. Thus in the dog (but not in the cat) the liver is the 'shock organ' and the main source from which histamine is released in peptone shock and anaphylaxis, and the same is true for shock produced by a histamine liberator. Moreover, a dog which has just recovered from peptone shock cannot be shocked again by treatment with either peptone or a histamine liberator. It may be significant that the licheniformins, which are among the most active histamine liberators we have examined, are polypeptides containing a high proportion of basic amino-acids; the pharmacologically active agents in peptone may have a similar constitution.

Rocha e Silva and his co-workers in a series of recent papers (Rocha e Silva, 1941, 1944; Dragstedt *et al.* 1942) have revived in a modified form the theory that the symptoms of anaphylaxis depend on the activation, consequent on the union of antigen and antibody, of some proteolytic enzyme, which then splits off histamine or other toxic substances from the tissue proteins to which they are bound. Such an activation has been demonstrated for the fibrinolytic enzyme of dog's plasma in anaphylactic and peptone shock (Rocha e Silva & Texeira, 1946; Rocha e Silva, Andrade & Texeira, 1946; Scroggie, Jaques & Rocha e Silva, 1947); and the striking resemblance of the shock produced by trypsin injections (Rocha e Silva, 1944) to peptone and anaphylactic shock

suggests that a generalized activation of certain proteases might acount for many of the changes observed in the two last-named conditions. We have not as yet studied the activity of plasma fibrinolysin or other proteases in dogs treated with histamine liberators, but the pharmacological similarity between these compounds and peptone suggests that such a study might be worth while. Besides the displacement hypothesis, therefore, the possibility must be considered that the histamine liberators act by unmasking tissue or plasma proteases. Our findings are so far indecisive on this point, which it should be possible to settle experimentally. We wish rather to draw attention to the fact, which we think of considerable significance, that there exists a large group of organic bases whose action reproduces the salient features of anaphylaxis and peptone shock.

SUMMARY

1. Many organic bases have the property, when given by vein to the cat, of producing a sudden fall of arterial pressure, the beginning of which is delayed for some 20–25 sec. after the injection.

2. Blood or plasma obtained during the period of lowered blood pressure contains a depressor substance whose action is manifested within a few seconds after injection. This substance has been identified pharmacologically as histamine, and it is released in amounts sufficient to account for the vascular effects of the bases which liberate it.

3. Among the bases found to liberate histamine were diamines, diamidines, diguanidines, diisothioureas, diquaternaries, some benzamidine derivatives, and licheniformin, an antibiotic polypeptide.

4. Such compounds elicit a typical triple response when injected into human skin. When continuously infused by vein into a cat they evoke a secretion of gastric juice.

5. In the dog these compounds likewise lower the blood pressure by releasing histamine in large amounts, and in addition they decrease or abolish the coagulability of the blood. The latter effect appears to be due to the liberation of heparin.

6. The liver is the main site of histamine release in the dog. In the cat, the main sites of the release are skin and muscle, and the liver is not involved.

7. The vascular effects of histamine liberators are reduced but usually not prevented by anti-histamine drugs. A variety of substances, including heparin, calcium salts and alkalis, also reduce these effects, apparently by interfering with the histamine release.

8. Possible mechanisms for the release of histamine by such compounds are considered, with particular reference to the similarity of their effects to the phenomena of anaphylaxis and peptone shock.

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